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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/563,484	08/03/2006	David Gutig	82498	1037

23685 7590 03/02/2011

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EXAMINER	
STRZELECKA, TERESA E	
ART UNIT	PAPER NUMBER
1637	
MAIL DATE	DELIVERY MODE
03/02/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/563,484	GUTIG, DAVID	
	Examiner	Art Unit	
	TERESA E. STRZELECKA	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 July 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *17-20 and 23-30* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16,21 and 22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on July 19, 2010 has been entered.

2. Claims 1-30 were previously pending, with claims 17-20 and 23-30 withdrawn from consideration. Applicant did not amend any claims. Claims 1-16, 21 and 22 will be examined.

3. Applicant's arguments were not considered to be persuasive in overcoming the previously presented rejections and are addressed in the "Response to Arguments" section below.

4. This office action contains new grounds for rejection.

Response to Arguments

5. Applicant's arguments filed July 19, 2010 have been fully considered but they are not persuasive.

A) Regarding the rejection of claims 1-13 under 35 U.S.C. 102(a) as being anticipated by Bransteitter et al., Applicant argues the following:

"Applicant respectfully disagrees with the Patent Office's line of reasoning. Bransteitter et al. does not teach or suggest treating DNA with AID to distinguish cytosine and 5-methylcytosine. In fact, the only comment in Bransteitter et al. that is even remotely related is an isolated comment in the paragraph bridging the left and right columns on page 4106 ("AID exhibits 10-fold higher specific activity on ssDNA for the deamination of dC → dU compared with 5-methylcytosine → T (Fig. 4b)." However, merely stating the differential activity of AID towards dC

and 5-methylcytosine does not teach or suggest using AID to distinguish cytosine and 5-methylcytosine. Moreover, nothing in Figs. 1 and 2 of Bransteitter et al. has anything to do with the differential activity of AID with respect to cytosine and 5-methylcytosine nor does anything anywhere in Bransteitter et al. teach or suggest using this property to determine the methylation status of cytosine bases in DNA. In fact, as noted above, Figs. 1 and 2 are completely silent about whether the DNA under investigation includes any methylated cytosines. To the extent that the Patent Office appears to be arguing that Bransteitter et al. detects dC deamination and that this fact necessarily results in a conclusion that a methyl group was present on the cytosine, Applicant notes that Bransteitter et al. does not teach or suggest using dC deamination to detect cytosine methylation. (Moreover, although not taught or suggested in Bransteitter et al., Applicant wishes to point out that dC deamination is more likely to suggest an unmethylated cytosine, as opposed to a methylated cytosine.)

In short, Bransteitter et al. does not recognize the methylation status of the DNA under investigation in its Figs. 1 and 2 nor does it teach or suggest that the differential activity of AID can be used to distinguish methylated and unmethylated cytosines. Consequently, it cannot be said that Bransteitter et al. teaches or suggests the concluding step of claim 1"

First, let us look at the claimed method steps. The claims require bringing the DNA into contact with cytidine deaminase, which deaminates cytidine and 5-methylcytidine at different rates, investigating the partially deaminated DNA sequence and concluding, from the presence or proportion of deaminated positions, the methylation status of DNA to be investigated. Applicant argues that Bransteitter et al. are not interested in the investigation of DNA methylation, but in the activity assay for the AID protein. However, the activity assay is based on the detection of

conversion (or lack thereof) of cytidine to uracil, i.e., if non-methylated cytidine is present in the sequence targeted, it is deaminated, therefore the final detection step in the assay determines whether the cytidine was methylated or not (see Figures 1 and 4b). Further, Bransteitter et al. specifically teach that AID deaminates methylated cytidines at a different rate than the unmethylated one (Fig. 4b).

In conclusion, Bransteitter et al. specifically teach detecting methylation status of cytidines by deaminating treatment with cytidine deaminase and specifically teach that the deamination reaction proceeds at a different rate for cytidines and methylated cytidines.

The rejection is maintained.

B) Regarding the rejection of claims 12-16, 21 and 22 under 35 U.S.C. 103(a) over Bransteitter et al. and Olek et al., Applicant argues that since claim 1 is not anticipated by Bransteitter et al., the rejection is improper. Arguments regarding the rejection of claim 1 were addressed above.

The rejection is maintained.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

7. Claims 1-13 are rejected under 35 U.S.C. 102(a) as being anticipated by Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS and in the previous office action).

Regarding claim 1, Bransteitter et al. teach a method for the detection of cytosine methylation in DNA (Abstract) comprising the steps of:

a) bringing the DNA to be investigated into contact with a cytidine deaminase, whereby the cytidine deaminase deaminates cytidine and 5-methylcytidine at different rates (page 4102, paragraphs 3-5; Fig. 1 and 4b; page 4106, fourth paragraph),

b) investigating the partially deaminated DNA with respect to its sequence (page 4102, last paragraph; page 4103, first and second paragraph), and

c) concluding from the presence or the proportion of deaminated positions the methylation status of the DNA to be investigated in said positions (Fig. 1; Fig. 2).

Regarding claim 2, Bransteitter et al. teach AID (page 4102, fourth paragraph).

Regarding claims 3 and 4, Bransteitter et al. teach single-stranded and partially-single stranded DNA (page 4102, third paragraph; Table 1).

Regarding claims 5-7, Bransteitter et al. teach single stranded regions being between 3 and 20 nucleotides long, between 5 and 12 nucleotides long and 9 nucleotides long (Table 1, page 4106).

Regarding claims 8 and 9, Bransteitter et al. teach oligomers between the length of 20 to 150 nucleotides and 35-60 nucleotides (Table 1, page 4106).

Regarding claims 10 and 11, Bransteitter et al. teach oligomers concentration of 100 nM (page 4102, fifth paragraph), anticipating the claimed ranges.

Regarding claims 12 and 13, Bransteitter et al. teach amplification of the deaminated fragment using a polymerase (page 4103, second paragraph).

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 12-16, 21 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS and in the previous office action) and Olek et al. (U.S. Patent No. 7,229,759 B2; cited in the previous office action).

A) Bransteitter et al. teach detection of the converted uracil residues using primer extension and ddA, but do not teach PCR or real-time PCR or using blocker oligonucleotides in the amplification reaction.

B) Regarding claims 12-14, 21 and 22, Olek et al. teach detection of deaminated cytosines resulting from bisulfite reaction using real-time PCR (col. 5, lines 37-53; col. 13, lines 45-59).

Regarding claim 15, Olek et al. teach methylation-specific primers (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31).

Regarding claim 16, Olek et al. teach using blocking oligonucleotides during amplification (col. 6, lines 3-20 and 38-67; col. 11, lines 29-49).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used the amplification methods of Olek et al. with blocking oligonucleotides to detect the converted cytidines in the method of Bransteitter et al. The motivation to do so is provided by Olek et al. (col. 13, lines 52-59 and col. 11, lines 67 and col. 12, lines 1-3):

"A particularly preferred variant of the method, however, is the simultaneous detection of qualifier positions and classifier positions in one experiment, which can be achieved by the use of TaqMan or LightCycler technology variants. Additional fluorescently labeled oligonucleotides are to be added to the oligonucleotides, which provide for a preferred amplification of the DNA to be investigated, and the change in fluorescence is measured during the PCR reaction. In principle, since the DNA to be investigated is amplified, information on the methylation status of different classifier CpG positions is obtained predominantly also directly from this change in fluorescence. Since different oligonucleotides are each preferably provided with different fluorescent dyes, a distinction of the change in fluorescence during the PCR is also possible, separately for different positions."

"If only one small group of CpGs is available and still a high amount of background DNA has to be blocked, it is therefore preferred that one part of this group of CpGs is covered by a methylation specific primer and the other part is covered by a methylation specific blocking probe, and the binding site of this non-extendible probe could ideally even overlap with the binding site of the primer. This way, high relative sensitivity, this means highly preferred amplification of the DNA to be analyzed while suppressing the background DNA, can be achieved with only a small group of CpGs as Qualifier positions."

10. Claims 1-16, 21 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olek et al. (U.S. Patent No. 7,229,759 B2; cited in the previous office action), as evidenced by Olek-2 et al. (Nuc. Acids Res., vol. 24, pp. 5064-5066 (1996), and Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS and in the previous office action).

A) Regarding claim 1, Olek et al. teach a method for detection of cytosine methylation in DNA comprising the steps of:

a) bringing the DNA to be investigated into contact with bisulfite (col. 5, lines 40-45; col. 9, lines 11-33),

b) investigating the partially deaminated DNA with respect to its sequence (col. 5, lines 46-49; col. 9, lines 34-67; col. 10, lines 1-59), and

c) concluding from the presence or the proportion of deaminated positions the methylation status of the DNA to be investigated in said positions (col. 5, lines 50-53; col. 10, lines 60-63).

Regarding claim 3, Olek et al. teach the DNA being in a single stranded form (col. 9, lines 22-24).

Regarding claims 4, 8, 9 and 15, Olek et al. teach methylation-specific primers (= oligomers) with lengths of 28 bp (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31; col. 19, lines 9-12).

Regarding claims 4-7, Olek et al. teach annealing primers to DNA during PCR, therefore they inherently teach single-stranded regions of different lengths (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31; col. 19, lines 9-12).

Regarding claims 10 and 11, Olek et al. teach primer concentrations of 500 nM (col. 24, line 6).

Regarding claims 12-14, 21 and 22, Olek et al. teach detection of deaminated cytosines resulting from bisulfite reaction using real-time PCR (col. 5, lines 37-53; col. 13, lines 45-59).

Regarding claim 15, Olek et al. teach methylation-specific primers (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31).

Regarding claim 16, Olek et al. teach using blocking oligonucleotides during amplification (col. 6, lines 3-20 and 38-67; col. 11, lines 29-49).

B) Olek et al. do not teach using cytidine deaminase to deaminate cytidines.

C) Regarding claim 1, Bransteitter et al. teach a method for the detection of cytosine methylation in DNA (Abstract) comprising the steps of:

a) bringing the DNA to be investigated into contact with a cytidine deaminase, whereby the cytidine deaminase deaminates cytidine and 5-methylcytidine at different rates (page 4102, paragraphs 3-5; Fig. 1 and 4b; page 4106, fourth paragraph),

b) investigating the partially deaminated DNA with respect to its sequence (page 4102, last paragraph; page 4103, first and second paragraph), and

c) concluding from the presence or the proportion of deaminated positions the methylation status of the DNA to be investigated in said positions (Fig. 1; Fig. 2).

Regarding claim 2, Bransteitter et al. teach AID (page 4102, fourth paragraph).

Regarding claims 3 and 4, Bransteitter et al. teach single-stranded and partially-single stranded DNA (page 4102, third paragraph; Table 1).

Regarding claims 5-7, Bransteitter et al. teach single stranded regions being between 3 and 20 nucleotides long, between 5 and 12 nucleotides long and 9 nucleotides long (Table 1, page 4106).

Regarding claims 8 and 9, Bransteitter et al. teach oligomers between the length of 20 to 150 nucleotides and 35-60 nucleotides (Table 1, page 4106).

Regarding claims 10 and 11, Bransteitter et al. teach oligomers concentration of 100 nM (page 4102, fifth paragraph), anticipating the claimed ranges.

Regarding claims 12 and 13, Bransteitter et al. teach amplification of the deaminated fragment using a polymerase (page 4103, second paragraph).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used cytidine deaminase of Bransteitter et al. to differentially deaminate cytidines

in the method of Olek et al. The motivation to do so would have been that deaminase treatment was accomplished in as little as 5 minutes (Bransteitter et al., page 4102, fifth paragraph) and did not require toxic chemicals such as sodium metabisulphite, the reaction with which lasts at least 4 hours at 50° C followed by 2. 5 hours of dialysis treatment (Olek-2 et al., page 5065, second and third paragraph). Therefore one of ordinary skill in the art would have recognized the advantages of using enzymatic deamination rather than chemically-induced one.

11. No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA E. STRZELECKA whose telephone number is (571)272-0789. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Teresa E Strzelecka

Primary Examiner
Art Unit 1637

/Teresa E Strzelecka/
Primary Examiner, Art Unit 1637
February 28, 2011